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(57) Abstract

The present invention comprises novel and modified peptides capable of inducing an HIV–1 specific immune response without antagonizing the cytotoxic T–cell activity in order to achieve an effective prophylactic and therapeutic vaccine against HIV. The peptides are based on conserved regions of HIV gag p24 proteins. Antigens in free– or carrier–bound form comprising at least one of the said peptides, vaccine compositions containing at least one of the antigens, immunoassay kits and a method of detecting antibodies induced by HIV or HIV specific peptides using such antigens, are described.

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HIV peptides, antigens, vaccine compositions, immunoassay kit and a method of detecting antibodies induced by HIV

The present invention relates to novel peptides based on conserved regions of HIV gag p24, antigens in free or carrier-bound form comprising at least one of the said peptides, vaccine compositions containing at least one of the antigens, immunoassay kits and a method of detecting antibodies, induced by human immunodeficiency virus (HIV) or HIV-specific peptides, using such antigens.

BACKGROUND

There is an urgent need to control the global epidemic of HIV infection and the development of a vaccine against HIV is one of the major objectives in AIDS research. In general vaccines should activate antigen presenting cells, overcome genetic restriction in T-cell responses and generate T- and B-memory cells. The variability of the viral population poses a further difficulty in obtaining an effective HIV vaccine. A break through in the ongoing attempts to develop a vaccine against AIDS has so far not been reported. It is now generally accepted that an induction of antigen-specific humoral and cell-mediated immunity is crucial for a development of an effective prophylactic and therapeutic vaccine. All three arms of the immune system including neutralizing antibodies; CD8+CTL and T-helper-1 (TH1) cells might be required for protective immunity to HIV. It is known that CTL can clear other viral infections (Ada, Immunol. Cell Biol., 72:447-454, 1994) and that CTL can lyse infected targets early in infection before viral progeny can be produced and released by cell lysis, Ada et al., supra.The focus has been on selection of antigens as well as on design and evaluation of different adjuvances. The antigens used in different *in vitro* and *in vivo* studies have been all from crude proteins to various synthetic peptides mainly from gp160 and to some extent from p24. A large number of studies have been done on the V3 loop of gp120. Induction of both B- and T-cell responses have been observed, however, it has been reported from an *in vitro* study that a peptide from the conserved region of gp41 have indicated infection enhancement (Bell S.J., et al., Clin. Exp. Immunol., 87 (1) : 37-45, (January 1992).

Naturally occurring HIV sequences in vaccine candidates are not capable of stimulating a stable immune response due to the viruses inherent ability to hide by changing the appearance of the epitopes presented on the cell surface of infected cells. The immune system is fooled to believe that a particular amino acid sequence is relevant when in fact the amino acids of importance is hidden.

A resent study of titers of antibodies against the gag p24 protein, has shown that slow progression towards development of AIDS is associated with high titers, while fast progression towards development of AIDS is associated with low titers. It is shown that persons with low p24 antibody titer develop significantly faster AIDS than persons with high p24 antibody titers (Zwart G., et al. Virology, 201, p. 285-93, June 1994), indicating that p24 can play a key role to control the development of AIDS.

New HIV p24 peptides are described in WO91/13360, wherein the peptides are used in a method of discriminating between a false and true diagnosed HIV-positive serum sample.

Johnson R.P., et al., The Journal of Immunology, Vol.147, p.1512-1521, No.5, September 1, 1991 describe an analysis of the fine specificity of gag-specific CTL-responses in three HIV-1 seropositive individuals, the gag-specific CTL-responses were found to be mediated by CD3+CD8+ lymphocytes which are HLA class I restricted.

EP-A-0 356 007 discloses antigenic determinants, in particular it relates to synthetic polypeptide sequences which are related to proteins present in the HIV-1 and which can be used as a basis for a potential vaccine against AIDS.

Rosenberg E.S. et al., Science, Vol.278, 21 November 1997, p.1447-1450 describe that virus specific CD4+ T helper lymphocytes are critical to the maintenance of effective immunity in a number of chronic viral infections, but are characteristically undetectable in chronic human immunodeficiency virus-type 1 (HIV-1) infection. HIV-1-specific proliferative responses to p24 were inversely related to viral load. They

conclude that the HIV-1-specific helper cells are likely to be important in immunotherapeutic interventions and vaccine development.

EP 0 230 222, EP 0 270 114, DE 37 11 016 and GB 2 188 639 all in the name of F. Hoffmann-La Roche & Co. Aktiengesellschaft concern recombinant expression and purification of an HTLVIII Gag/Env gene protein or fusionproteins. The proteins consisting of native sequences can be purified to homogeneity and used as a basis for diagnostic tests for detection of antibodies against viruses associated with AIDS. The gag/env protein may also be formulated for use as a vaccine for protection against AIDS through prophylactic immunization.

From a diagnostic and therapeutic point of view, the major problems with using p24 as part of an assay or therapy is associated with the high number of epitopes on p24 which stimulates production of a large number of antibodies with poor specificity, which through repeated boostering on potential mutated sequences can create autoantibodies (Autoantibodies to the alfa/beta T-cell receptors in HIV infection; dysregulation and mimicry. Lake D.F., et al., Proc. Natl. Acad. Sci. USA, (23) : 10849-53, Nov. 8 1994). Further, it is reported that the p24 antibody titer does not reach the same high levels as for the envelope proteins (gp120 and gp41). Normally antibodies to p24 are developed in the very early phase of the infection, but the titer is fairly quickly stabilized after the initial infection period. Later the p24 titer is gradually decreasing while the opposite happens with gp160. These findings can also be seen in relation to recent reports stating that cytotoxic T-cell activity is antagonized by naturally occurring HIV-1 gag variants (Klenerman P., et al., Nature, 2:369 (6479), p. 355, 2 June 1994). This can be one of the reasons why a rapid stabilization of the p24 titer is seen and why it later starts to decrease.

Based on the above background data, we decided to investigate the possibility of designing novel synthetic peptides which can mimic the p24 epitope without antagonizing the cytotoxic T-cell activity, in order to meet the need for an effective prophylactic and therapeutic vaccine.

The intital work was based on one epitope which was published by Korber B., et al., Human Retroviruses and AIDS 1997 Eds.Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM. The amino acid sequence of this epitope (203-222) was:

K A L G P G A T L E E M M T A C Q G V G
R R M R T K S I K D L S S S R R
G V R V
S A R
S E
Q Q

The one letter as well as the three letter codes defining the amino acids in the sequences given throughout this specification are in accordance with International standards and given in textbooks, for instance Lehninger A.L., «Principles of Biochemistry», Worth Publishers Inc., New York, 1982. The aminoacids given below the head sequence represent the natural variation of the sequence. An initial study of a sequence containing this modified epitope was conducted on the sequence :

A N P D C K Q I L K S L G P G A T L E E X X T A C Q G V G - NH_2

wherein X indicates 2-aminohexanoic acid and the cysteine residues are in an oxidized state, i.e. are forming an intrachain disulphide bridge. The results (unpublished) from studies using this peptide as part of a diagnostic kit showed that the specificity became 87% (n=279) on a preselected panel of African sera. The sensitivity was surprisingly 100% on a panel of HIV-1 positive sera including HIV-1 subtype O sera, which is quite different from the other subtypes.

In order to improve specificity, i.e. define the amino acids which contribute to a pure non-crossreacting antibody response, a similar study was applied to a significantly shorter and further modified peptide:

L I W G A T C Q E H X T A C Q G V G - NH_2

wherein X has the above mentioned meaning and the cysteine residues are forming an intrachain disulphide bridge.

The results from this study showed that the specificity of the assay increased to 96%, and (n=293) which is similar to the specificity obtained in the assay without using the p24 peptide. With a specificity of 87% to the assay where the first peptide was included, it would be likely that the peptide would induce immune response to more than one epitope since it was recognized by unspecific antibodies, if it was used as a vaccine candidate. The latter, however, show that the peptide sequence is picking up an immune response which is unique to HIV-1. Consequently, if a sequence based on this is used as an antigen in a vaccine candidate, it would most likely boost an unique immune response to HIV-1.

To further increase the number of T-cell epitopes and reduce the probability for development of escape mutants three additional peptide sequences were based on the following three sequences from residues 264-284, 253-271 and 166-186, respectively published in Human Retroviruses and AIDS 1997; A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences. Eds.Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos :

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R W I I L G L N K I V R M Y S P T S I L D
K G V V M         M   K     C   V G     E
D   M   V         V         Q   I       G
                                S
                                A
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N N P P I P V G E I Y K R W I I L G L
S   Q A V       K D M L R K G M V M
G   G S N         K V     D   V   V
H     G T
A
P
```

and

P	E	V	I	P	M	F	S	A	L	S	E	G	A	T	P	Q	D	L	N	T
R	I	T	T	T	L	T	E		A	D			I	S	Y	N	I	Y	M
	L	N			A	L							V		H		V		I
					M								L						A
																			V

Several modified peptides have been synthesized in order to determine unique sequences which are both specific and sensitive towards HIV-1.

DESCRIPTION OF THE INVENTION

The peptides according to the invention are originating from the four different conserved areas of the HIV-1 core protein p24 which are described above, having the properties of maintaining the uniqueness (sensitivity and specificity) of the HIV-1-epitope. Further the new peptides according to the invention possess no recognized cytotoxic T lymphocyte (CTL) antagonistic effect and shall have at least one potential CTL epitope.

The peptides, according to the invention, which have met the above criteria are selected from the following groups ;

$Xaa_1\ Xaa_2\ Xaa_3\ Xaa_4\ Xaa_5\ Xaa_6$ Ala $Xaa_8\ Xaa_9$ Gln Thr Pro Trp $Xaa_{14}\ Xaa_{15}\ Xaa_{16}\ Xaa_{17}$ Xaa_{18} Val Xaa_{20} (SEQ ID NO : 1)

wherein the amino acids of the chain could have the following meanings ;
Xaa in position 1 of the peptide derivate is Lys or Arg,
Xaa in position 2 is Ala, Gly, Ser or Arg,
Xaa in position 3 is Leu or Met,
Xaa in position 4 is Gly or Arg,
Xaa in position 5 is Pro, Thr, Val, Ser, Gln or Ala,
Xaa in position 6 is Gly, Ala, Lys, Arg, Gln or Glu,
Xaa in position 8 is Thr or Ser,

Xaa in position 9 is Leu or Ile ,
Xaa in position 14 is Thr, Ser or Val,
Xaa in position 15 is Ala or Ser,
Xaa in position 16 is Cys or Ser,
Xaa in position 17 is Gln or Leu
Xaa in position 18 is Gly, Glu or Arg,
Xaa in position 20 is Gly or Arg,

the peptide comprises at least nine consecutive amino acids of the sequence of SEQ ID NO : 1,

$Xaa_1\ Xaa_2\ Xaa_3\ Xaa_4\ Xaa_5$ Gly Leu Asn Pro Leu Val $[Gly]_n\ Xaa_{12}\ Xaa_{13}$ Tyr Xaa_{15} Pro $Xaa_{17}\ Xaa_{18}$ Ile Leu $Xaa_{21}\ Xaa_{22}$ (SEQ ID NO : 4)

wherein the amino acids of the chain have the following meaning;
Xaa in position 1 is Arg, Lys, Asp or none
Xaa in position 2 is Trp, Gly, Lys or Arg,
Xaa in position 3 is Ile, Leu, Val or Met
Xaa in position 4 is Ile, Val or Leu
Xaa in position 5 Leu, Met, Val or Pro
Xaa in position 12 is Arg, Lys
Xaa in postion 13 is Met or Leu,
Xaa in position 15 is Ser, Cys or Gln,
Xaa in position 17 is Thr, Val, Ile, Ser or Ala,
Xaa in position 18 is Ser, Gly or Thr,
Xaa in position 21 is Asp, Glu, Cys or Gly,
Xaa in position 22 is Gly or none
wherein the sequence of SEQ ID NO : 4 comprises at least six consecutive amino acids and n = 0,1,2 or 3,

$Xaa_1\ Xaa_2\ Xaa_3$ Pro Ile Pro $Xaa_7\ Xaa_8\ Xaa_9\ Xaa_{10}\ Xaa_{11}\ Xaa_{12}$ $[Gly]_n$ $Xaa_{13}\ Xaa_{14}\ Xaa_{15}$ $Xaa_{16}\ Xaa_{17}\ Xaa_{18}\ Xaa_{19}\ Xaa_{20}\ Xaa_{21}\ Xaa_{22}\ Xaa_{23}\ Xaa_{24}$ (SEQ ID NO : 9)

wherein Xaa in position 1 is Asn, Ser, Gly, His, Ala, Pro, Arg or none
Xaa in position 2 is Asn, Ala or Lys
Xaa in position 3 is Pro, Gln, Gly, Ile or Leu
Xaa in position 7 is Val or Ala
Xaa in position 8 is Gly or Lys
Xaa in position 9 is Glu, Asp, Lys, Phe or Thr
Xaa in position 10 is Ile, Met, Val or Leu
Xaa in position 11 is Tyr, Leu or none
Xaa in position 12 is Ser or none
Xaa in position 13 is Arg or none
Xaa in position 14 is Asp, Arg, Trp, Ala or none
Xaa in position 15 is Ile or none
Xaa in position 16 is Tyr or none
Xaa in position 17 is Lys or Arg
Xaa in position 18 is Arg, Lys or Asp
Xaa in position 19 is Trp or Gly
Xaa in position 20 is Ile, Met, Val, Gln or Ala
Xaa in position 21 is Ile, Val or Ala
Xaa in position 22 is Leu, Met or Val
Xaa in position 23 is Gly or Cys
Xaa in position 24 is Leu or none

wherein the sequence of SEQ ID NO : 9 consists of at least six consecutive amino acids and n = 1,2 or 3,

$Xaa_1\ Xaa_2$ Ile Ile $Xaa_5\ Xaa_6\ Xaa_7\ Xaa_8\ Xaa_9$ Leu Xaa_{11} $[Gly]_n$ $[Arg]_m$ $Xaa_{12}\ Xaa_{13}\ Xaa_{14}\ Xaa_{15}\ Xaa_{16}\ Xaa_{17}\ Xaa_{18}\ Xaa_{19}\ Xaa_{20}\ Xaa_{21}\ Xaa_{22}\ Xaa_{23}\ Xaa_{24}\ Xaa_{25}$ (SEQ ID NO : 15)

wherein the Xaa in position 1 is Pro, Lys, Arg or none
Xaa in position 2 is Glu, Arg, Phe or Lys
Xaa in position 5 is Pro or Thr
Xaa in position 6 is Met, Thr or Nleu
Xaa in position 7 is Phe or Leu

Xaa in position 8 is Ser, Thr, Ala or Met
Xaa in position 9 is Ala, Glu or Leu
Xaa in position 11 is Ser or none
Xaa in position 12 is Ala, Arg or none
Xaa in position 13 is Ile, Leu or none
Xaa in position 14 is Ser, Ala, Leu or none
Xaa in position 15 is Tyr, Glu or Asp
Xaa in position 16 is Gly or Asp
Xaa in position 17 is Ala or Leu
Xaa in position 18 is Thr, Ile, Val, Leu or Asn,
Xaa in position 19 is Pro, Thr or Ser
Xaa in position 20 is Tyr, Phe, Nleu, His or Gln
Xaa in position 21 is Asp, Asn, Leu or Ala
Xaa in position 22 is Leu, Ile, Val or Asn
Xaa in position 23 is Asn, Tyr, Cys or Gly
Xaa in position 24 is Thr, Met, Ile, Ala, Val or none
Xaa in postion 25 is Gly or none
wherein the sequence of SEQ ID NO : 15 consists of at least six consecutive amino acids, n = 1,2 or 3 and m=0,1,2 or 3,

the terminal ends of the sequences may be free carboxyl- or amino groups, amides, acyls, acetyls or salts thereof,
two or more of the Cys residues may form part of an intrachain- or interchain disulphide binding, a -S-$(CH_2)_p$-S- or a - $(CH_2)_p$-bridge wherein p = 1-8, optionally intervened by one or more heteroatoms such as O, N or S and/or the said peptide sequences are immobilized to a solid support.

The new peptide sequences have the potential to serve as a good antigen wherein the antigen comprises at least one peptide selected from the group of sequences of SEQ ID NO : 1, SEQ ID NO : 4, SEQ ID NO : 9 or SEQ ID NO : 15 . The antigenicity may be adapted through adjusting the ratio or concentration of different peptides or size of the peptides by for instance dimerisation or polymerisation and/or immobilisation to a solid phase. The antigen comprises two or more polypeptide sequences, according to the

invention, which are either linked by a bridge for instance a disulphide bridge between the Cys residues of the chains or bridges like C_1-C_8 alkylen possibly intervened by one or more heteroatoms like O, S, or N or preferably they are unlinked. The chains may be immobilized to a solid phase in monomeric, dimeric or oligomeric forms. Further amino acids may be added to the ends in order to achieve an «arm» to facilitate immobilization.

All amino acids in the peptides of the invention can be in both D- or L-form, although the naturally occurring L- form is preferred.

The C- and N-terminal ends of the peptide sequences could deviate from the natural sequences by modification of the terminal NH_2-group and/or COOH-group, they may for instance be acylated, acetylated, amidated or modified to provide a binding site for a carrier or another molecule.

The peptides according to the invention are consisting of 6 to 50 amino acids, preferably between 10 and 30 amino acids. They are covering all natural variation of amino acids in the identified positions.

The polypeptide antigen according to the invention is either in a free or in a carrier-bound form. The carrier or solid phase to which the peptide is optionally bound can be selected from a vide variety of known carriers. It should be selected with regard to the intended use of the immobilized polypeptide as a diagnostic antigen or as an immunizing component in a vaccine.

Examples of carriers that can be used for e.g. diagnostic purposes are magnetic beads or latex of co-polymers such as styrene-divinyl benzene, hydroxylated styrene-divinyl benzene, polystyrene, carboxylated polystyrene, beads of carbon black, non-activated or polystyrene or polyvinyl chloride activated glass, epoxy-activated porous magnetic glass , gelatine or polysaccharide particles or other protein particles, red blood cells, mono- or polyclonal antibodies or fab fragments of such antibodies.

According to a further embodiment of the present invention, the antigens may form part of a vaccine possibly combined with carriers, adjuvants or combined with other immunostimulating elements such as canarypox virus carrying the *env* gene. Examples of carriers and/or adjuvants for vaccine purposes are other proteins such as human or bovine serum albumin and keyhole limpet haemocyanin. Immunostimulatory materials may be divided into three groups; adjuvants, carriers for antigens and vehicles. Examples of adjuvants include aluminum hydroxyd, aluminum salts, saponin, muramyl di- and tri-peptides, monophosphoryl lipid A, *B.pertussis* and various cytokines including the Th1 cytokine IL-12 and IL-1. A number of protein toxins can be used to carry passenger proteins across cellular membranes into the cytosol, which are useful in developing CTL vaccines. Carriers include bacterial toxoids such as inactivated tetanus and cholera toxins, genetically detoxified bacterial toxins such as heat labile enterotoxin from *E.coli*, fatty acids, live vectors such as polio chimeras and hybrid proteins that form particulates for example yeast retrotransposon hybrid TY particles and HBcAg particles. Vehicles which are frequently occurring components in modern vaccines are consisting of mineral oil emulsion, Freunds complete and incomplete adjuvant, vegetable oil emulsions, nonionic block co-polymer surfactants, squalene or squalane, liposomes and biodegradable microspheres. Two novel adjuvants which possess significant potential for the development of new vaccines include an oil-in-water microemulsion (MF59) and polymeric microparticles. Any substance that can enhance the immunogenicity of the antigen may be used and several further alternatives of carriers or adjuvants are given in the US or European Pharmacopoeia.

A suitable formulation of the antigen for immunostimulatory uses may also comprise interferons such as INF-γ, antiviral chemokines or haematopoietic growth factors such as granulocyte macrophage growth factor.

Another approach in order to enhance the stimulation and absorption in for instance the intestine is to administer the peptides of the invention, with small peptides such as di- tri- or tetra peptides. These peptides can be administered in addition to or in combination with the peptides of the invention. Preferably the peptides are administered together with the tripeptide YGG, consisting of amino acids in the D- or L-forms, preferably in the D-form.

Recent approaches to non-parenteral delivery of vaccines, for instance via mucosa include; gene fusion technology to create non-toxic derivatives of mucosal adjuvants, genetically inactivated antigens with a deletion in an essential gene, coexpression of an antigen and a specific cytokine that is important in the modulation and control of a mucosal immune response, and genetic material itself that would allow DNA or RNA uptake and its endogenous expression in the host's cells.

One approach for developing durable responses where cell-mediated immunity is required, is to vaccinate with plasmid DNA encoding one or more specific antigen(s).

In order to protect against HIV infection, vaccines should induce both mucosal and systemic immune responses and could be administered by any convenient route, parenterally or non-parenterally, such as subcutanously, intracutanously, intravenously, intramuscularly, perorally, mucosally or intranasally for example.

In a preferred embodiment of the vaccine according to the present invention it comprises antigens containing the peptides of the SEQ ID NO : 1, 4, 9 and 15, more preferred the peptides occur in the ratio 1:1:1:1.

In a further preferred embodiment the vaccine composition contains the antigens ;

R A L G P A A T L Q T P W T A S L G V G - NH_2 (SEQ ID NO : 3)

R W L L L G L N P L V G G G R L Y S P T S I L G - NH_2 (SEQ ID NO : 6)

R A I P I P A G T L L S G G G R A I Y K R T A I L G - NH_2 (SEQ ID NO : 11)

and

R F I I P N I F T A L S G G R R A L L Y G A T P Y A I G - NH_2 (SEQ ID NO : 18).

One of the sequences contains a B-cell epitope and will activate the humoral immune system, whereas the other sequences contribute with CTL-epitopes and the amino acid changes implemented within the frame of the CTL-epitope are designed to achieve enhanced binding. Other amino acid changes have been conducted in order to facilitate the synthesis of the peptide and/or increase the solubility of the peptide.

A method for detecting antibodies, induced by HIV-1 or HIV-1 specific peptides or proteins, in a sample of body fluid using the present antigens is a further embodiment of the invention. Also immunoassay kit designed for this detection and antibodies capable of selectively reacting with the said antigens are encompassed by the present invention.

DESCRIPTION OF THE PREPARATION OF THE PEPTIDES

The peptides of the invention can be produced by any known method of producing a linear amino acid sequence, such as recombinant DNA techniques. A nucleic acid sequence which encodes a peptide of the invention or a multimer of the said peptides, is introduced into an expression vector. Suitable expression vectors are for instance plasmids, cosmids, viruses and YAC (yeast artifical chromosome) which comprise necessary control regions for replication and expression. The expression vector may be stimulated to expression in a host cell. Suitable host cells are for example bacteria, yeast cells and mammal cells. Such techniques are well known in the art and described for instance by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989. Other well-known techniques are degradation or synthesis by coupling of one amino acid residue to the next one in liquid phase or preferably on a solid phase (resin) for instance by the so-called Merrifield synthesis. See for instance Barany and Merrifield in the Peptides, Analysis, Synthesis, Biology, Vol.2, E. Gross and Meinhofer, Ed. (Acad.Press, N.Y., 1980), Kneib-Coronier and Mullen Int. J. Peptide Protein Res.,30, p.705-739 (1987) and Fields and Noble Int.J.Peptide Protein Res., 35, p.161-214 (1990).

In case a linked or cyclic peptide is desired, the amino acid sequence is subjected to a chemical oxidation step in order to cyclize or link the two cysteine residues within one or between two peptide sequences, when the appropriate linear amino acid sequences are synthesized, see Akaji et al., Tetrahedron Letter, 33, 8, p.1073-1076, 1992.

GENERAL DESCRIPTION OF SYNTHESIS

All peptide derivatives prepared in the Examples given below were synthesized on a Milligen 9050 Peptide Synthesizer using a standard program. The resin used was Tenta Gel P RAM with a theoretical loading of 0,20 meq/g (RAPP POLYMERE GmbH, Tübingen). The final product of the synthesis was dried *in vacuo* overnight. The peptide was then cleaved from the resin by treatment with 90% trifluoroacetic acid in the presence of ethandithiol (5%) and water (5%) as scavengers (1,5 hours at RT). Then the resin was filtered and washed on filter with additional trifluoroacetic acid (100%) (2 x 20 ml). The combined filtrates were evaporated *in vacuo* (water bath at RT) and the residue was triturated with ethyl ether (200 ml) and the precipitated product filtered off. The solid was promptly dissolved on filter with glacial acetic acid (100 ml) and added to 1,5 l of 20% acetic acid in methanol and treated with 0,1 M solution of iodine in methanol until a faint brown colour remained. Then Dowex 1 x 8 ion exchange in acetate form (15g) (Bio-Rad, Richmond, CA) was added and the mixture filtered. The filtrate was evaporated and the residue freeze-dried from acetic acid. The product was then purified by reversed phase liquid chromatography on a column filled with Kromasil® 100 - 5 C8 (EKA Nobel, Surte, Sweden) in a suitable system containing acetonitrile in 0,1 % trifluoroacetic acid water solution. The samples collected from the column were analyzed by analytical high performance liquid chromatography (HPLC) (Beckman System Gold, USA) equipped with a Kromasil® 100 - 5 C8 Column (EKA Nobel, Surte, Sweden). Fractions containing pure substance were pooled, the solvent was evaporated and the product freeze-dried from acetic acid. The final HPLC analysis was performed on final product, and the structure of the peptide was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

All amino acids used during the synthesis were L-amino acids and they were protected with a fluorenylmethoxy-carbonyl group at the α-amino function. The side chains were protected as follows :

Cys (Trt), Gln(Trt), Glu(OtBu), Thr(tBu).

The abbreviations, within the brackets are :

Trt = triphenylmethyl

t-Bu = tert. Butyl

OtBu = tert. Butylester

The amino acid derivatives was supplied by Bachem AG, Switzerland.

EXAMPLE 1

Preparation of K A L G P G A T L Q T P W T A C Q G V G - NH_2 (SEQ ID NO : 2).

The peptide was synthesized in amide form, from corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC): 87 %

EXAMPLE 2

Preparation of R A L G P A A T L Q T P W T A S L G V G (SEQ ID NO : 3).

The peptide was synthesized in amide form, from corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC): more than 95%

Molecular weight (free base): 1966

Molecular formula : $C_{88}H_{144}O_{25}N_{26}$

EXAMPLE 3

Preparation of W I I P G L N P L V G G G K L Y S P T S I L C G - NH_2 (SEQ ID NO : 5).

The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : 95%

Mass spectral analysis : Theoretical molecular weight : 2454.9

Experimental molecular weight : 2454.8 ES+

EXAMPLE 4

Preparation of R W L L L G L N P L V G G G R L Y S P T S I L G (SEQ ID NO : 6). The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 95 %

Molecular weight (free base) : 2552

Molecular formula : $C_{119}H_{195}O_{29}N_{33}$

EXAMPLE 5

Preparation of K I L L G L N P L V G G G R L Y S P T S I L G (SEQ ID NO : 7) , R L L L G L N P L V G G G R L Y S P T T I L G (SEQ ID NO : 8) and N I P I P V G D I Y G G G D I Y K R W Q A L C L (SEQ ID NO : 24). The peptides are synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity are determined by HPLC analysis and the structures are confirmed by amino acid analysis and mass spectrometry (LDI-MS).

EXAMPLE 6

Preparation of R N I P I P V G D I Y G G G D I Y K R W Q A L C L (SEQ ID NO : 10). The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : 85 %

Mass spectral analysis : Theoretical molecular weight : 2817.3

Experimental molecular weight : 2813.7 ES+

EXAMPLE 7

Preparation of R A I P I P A G T L L S G G G R A I Y K R W A I L G (SEQ ID NO : 11). The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC

analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 95 %

Molecular weight (free base) : 2707

Molecular formula : $C_{125}H_{208}O_{29}N_{38}$

EXAMPLE 8

Preparation of A L P I PA G F I Y G G G R I Y K R W Q A L G (SEQ ID NO : 12), K I P I P V G F I G G G W I Y K R W A I L G (SEQ ID NO : 13) and K I P I P V G T L L S G G G R I Y K R W A I L G (SEQ ID NO : 14). The peptides are synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity are determined by HPLC analysis and the structures are confirmed by amino acid analysis and mass spectrometry (LDI-MS).

EXAMPLE 9

Preparation of K F I I P NI F S A L G G A I S Y D L N T NI L N C I (SEQ ID NO : 16). The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. NI in the sequence is Norleucine. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 80 %

Mass spectral analysis : Theoretical molecular weight : 2783.3

Experimental molecular weight : 2783.3 ES+

EXAMPLE 10

Preparation of K F I I P NI F S A LS G G G A I S Y D L N T F L N C I G (SEQ ID NO : 17).

The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. NI in the sequence is Norleucine. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 80 %

Mass spectral analysis : Theoretical molecular weight : 2932.4

Experimental molecular weight : 2931.8 ES+

EXAMPLE 11

Preparation of R F I I P NI F T A L S G G R R A L L Y G A T P Y A I G (SEQ ID NO : 18).

The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. NI in the sequence is Norleucine. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Purity (HPLC) : more than 95 %

Molecular weight (free base) : 2894

Molecular formula : $C_{137}H_{217}O_{32}N_{37}$

EXAMPLE 12

Preparation of K I I P NI F S A L G G G R L L Y G A T P Y A I G (SEQ ID NO : 19), R I I P NI F T A L S G G G R L L Y G A T P Y A I G (SEQ ID NO : 20) and W I I P NI F S A L G G A I S Y D L N T NI L N C I (SEQ ID NO : 25). The peptides are synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity are determined by HPLC analysis and the structures are confirmed by amino acid analysis and mass spectrometry (LDI-MS).

EXAMPLE 13

Dimerisation via disulphide bridge.

The peptide sequences of the Examples 1 and 3 were linked via an oxidation step to form a dipeptide wherein the cysteine residues formed a disulphide bridge. The bridge was formed in either ways;

A) Oxidation with I_2 Equal amounts of the peptides were dissolved in acetic acid/methanol (1:4) and 0.1 M I_2 in methanol was added yielding a mixture of the dimer.

or

B) Oxidation via [Cys(Spy)^{16}]-SEQ ID NO : 2. 2,3mM of the peptide of SEQ ID NO : 2 dissolved in 2 M AcOH (aq) and 2-propanol (1:1) was treated with 2,2 dithiodipyridin (3 eqv) to yield [Cys(Spy)^{16}]-SEQ ID NO : 2. Equal amounts of [Cys(Spy)^{16}]-SEQ ID NO : 2

and peptide of SEQ ID NO : 5 were dissolved in 10 mM NH_4Oac (aq pH=6, 5) and methanol (5:2) to yield the dimer of SEQ ID NO : 21.

The purity of the peptide was determined by HPLC analysis and the peptide structure was confirmed by amino acid analysis. The peptide content (aminoacid free base) was 80%,
Purity (HPLC) : 92%.

EXAMPLE 14

A vaccine comprising the peptides of the SEQ ID NO : 3, 6, 11 and 18 was prepared. The freeze-dried peptides were dissolved in sterile water at a final concentration of 4 mg/ml. The final salt concentration was 0,9 %. A preparation of a granulocyte-macrophage-colony stimulating factor (GM-CSF) was also prepared, according to the manufacturers directions for use, to a final concentration of 0.3 mg/ml. The two solutions are administered intracutaneously. A typical injection dose is 100 µl.

EXAMPLE 15

An antigen solution or suspension is mixed with equal parts of Freund`s adjuvant of Behring, complete or incomplete, and is then finely emulsified by being drawn up into, and vigurously pressed out of, an injection syringe, or with a homogenator. The emulsion should remain stable for at least 30 minutes. The antigen-adjuvant emulsions is best injected subcutaneously as a depot.

EXAMPLE 16

Toxicity data.

The dipeptide of Example 13 was diluted in 0,9% NaCl to a test solution concentration of 4 mg/ml. The peptide was administered by injection to NMFI female mice in a dose of 100 µg per kg bodyweight. No toxicological effects were observed and the peptide was deemed not toxic.

Toxicity studies were performed in mice and rats on the peptide composition of the vaccine in Example 14. The mouse was selected for the study to provide comparative data from a second commonly used rodent species. The test substance was a mixture

of four peptides supplied as one vial containing lyophilised material for reconstitution with physiological saline, and dose levels were expressed in terms of total peptide load. The individual peptides was present in ratio 1:1:1:1 giving dose levels of each peptide of 0.0075 mg/kg body weight, 0.075 mg/kg body weight and 0.75 mg/kg body weight, which are up to 500 fold the intended human dose. The test animals were divided into four groups of ten animals each (five males and five females); a saline control group and groups for low, intermediate and high doses. The test composition was administered once, by intravenous infusion into a tail vein at a dose rate of 3 ml/minute. The animals were killed at day 15 and 16 by intraperitoneal injection of sodium pentobarbitone.

The results of these studies indicated that the dose levels administered to the mice and rats elicited no adverse reactions and that the no effect level was in excess of 3 mg/kg.

EXAMPLE 17

Immunoassay for detection of antibodies induced by HIV-1.

The magnetic particle reagents are to be prepared according to the manufacturers recommended protocol. Dynal AS, is the manufacturer of the Dynabeads, which are employed. The magnetic particles coated with ligand are called Reagent 1. A peptide according to the invention is covalently coupled to the pre-activated surface of the magnetic particles. It is also possible to physically absorb the peptide to the surface of the magnetic particles. The concentration of particles in Reagent 1 is within the range from 1 mg/ml to 15 mg/ml. The particle size varies between 0,2 μm to 15 μm. The concentration of peptides is within the range from 0,01 mg/mg particle to 1 mg/mg particle.

The anti human Ig Alkaline Phosphatase (AP) conjugated antibody reagent is prepared according to the recommended protocol of Dako AS. This protocol is a standard procedure in this field. This reagent is called Reagent 2.

The substrate solution phenolphtalein-monophosphate is to be prepared according to the recommended protocol of Fluka AG. This protocol is a standard procedure in this field. The substrate solution is called Reagent 3.

The washing and incubation buffer which is used is standard 0,05M tris-base buffer with the following additional compounds; Tween 20 (0,01% to 0,1%), glycerol (0,1% to 10%) and sodium chloride (0,2% to 0,1%).

The assay procedure comprises an incubation step wherein 1 drop of Reagent 1 is mixed with 2 drops of washing buffer in each well. After mixing, 30 µl of sample is added and the solution is incubated for 5 minutes. The magnetic particles can be trapped by a magnet and the liquid removed, before the magnet is separated. Then the wells are washed twice in 4 drops of washing solution, before incubation with Reagent 2. 1 drop of Reagent 2 is added with 2 drops of washing buffer and the solution is incubated for 5 minutes. The magnetic particles can be trapped by a magnet and the liquid removed, before the magnet is separated. Then the washing step is repeated before incubation with Reagent 3. 2 drops of Reagent 3 is added to each well and the solution is incubated for 3 minutes. The results can be read against a white background. Positive results are red (3+ = strong red) whereas negative results are clearly light yellow/brown solutions as obtained in the negative control.

The immunoassay kit could be used in detection of antibodies, induced either by HIV virus or HIV-specific peptides or proteins, for instance the peptides of the present invention.

The above Examples are only meant as illustrating the invention. It must be understood that a person skilled in the art can modify the peptides, antigens and vaccines herein described without deviating from the concept and scope of this invention as set forth in the claims.

The polypeptides of the invention can be used in a combination of at least one peptide selected from each group of sequences, SEQ ID NO : 1, SEQ ID NO : 4, SEQ ID NO : 9 and SEQ ID NO : 15 to form antigens and the the active principle of a prophylactic or therapeutic vaccine intended to provide protection against the human immunodeficiency virus type 1 (HIV-1). The vaccine may include compounds having beneficial effects in protecting or stimulating the host's immune system (human being or vertebrate animal) for instance interleukins, interferons, granulocyte macrophage growth factors, haematopoietic growth factors or similar. Preferably the vaccine

composition further contain an adjuvant or vehicle, more preferably the adjuvant or vehicle is Monophosphoryl Lipid A (MPL ®) possibly with alum, Freund's adjuvant (complete or incomplete) or aluminum hydroxyd. The optimal amount of adjuvant/vehicle will depend on the type(s) which is chosen.

The peptide or vaccine formulation can be freeze-dried prior to storage. The vaccine may be stored preferably at low temperature, in ampoules containing one or more dosage units, ready for use. A typical dosage unit of the peptide according to the invention is within the concentration range : 1 μg-1mg per kg bodyweight, preferably within 2 μg-0.15 mg per kg body weight. Persons skilled in the art will appreciate that a suitable dose will depend on the body weight of the pasient, the type of disease, severity of condition, administration route and several other factors. The vaccine might be administered up to twelve times and through injection, typically it will be administered about three times. In preparation of an injection solution the peptides are dissolved in sterile sodium chloride solution at a final concentration of 1 mg/ml per peptide and 0,9% sodium chloride. Typically an injection volume is 100 μl to 200 μl (2 x 100 μl). The peptide is preferably co-administered with a suitable adjuvant and/or a granulocyte-macrophage growth factor for instance Leucomax® «Shering Plough». Suitable administration may be intracutane, subcutane, intravenous, peroral, intramuscular, intranasal, mucosal or any other suitable route. Booster administrations may be required in order to maintain protection. For persons skilled in the art it will be understood that the vaccine compositions according to the invention are useful not only in prevention of infection, but also in treatment of infection.

PATENT CLAIMS

1. Peptide characterized in that it comprises at least one amino acid sequence selected from the groups of amino acid sequences :

$Xaa_1\ Xaa_2 Xaa_3 Xaa_4\ Xaa_5\ Xaa_6$ Ala $Xaa_8\ Xaa_9$ Gln Thr Pro Trp $Xaa_{14} Xaa_{15}\ Xaa_{16}\ Xaa_{17}$ Xaa_{18} Val Xaa_{20} (SEQ ID NO : 1)

wherein the amino acids of the chain could have the following meanings ;
Xaa in position 1 of the peptide derivate is Lys or Arg,
Xaa in position 2 is Ala, Gly, Ser or Arg,
Xaa in position 3 is Leu or Met,
Xaa in position 4 is Gly or Arg,
Xaa in position 5 is Pro, Thr, Val, Ser, Gln or Ala,
Xaa in position 6 is Gly, Ala, Lys, Arg, Gln or Glu,
Xaa in position 8 is Thr or Ser,
Xaa in position 9 is Leu or Ile ,
Xaa in position 14 is Thr, Ser or Val,
Xaa in position 15 is Ala or Ser,
Xaa in position 16 is Cys or Ser,
Xaa in position 17 is Gln or Leu
Xaa in position 18 is Gly, Glu or Arg,
Xaa in position 20 is Gly or Arg,
the peptide comprises at least nine consecutive amino acids of the sequence of SEQ ID NO : 1,

$Xaa_1 Xaa_2\ Xaa_3\ Xaa_4\ Xaa_5$ Gly Leu Asn Pro Leu Val $[Gly]_n Xaa_{12} Xaa_{13}$ Tyr Xaa_{15} Pro $Xaa_{17} Xaa_{18}$ Ile Leu $Xaa_{21}\ Xaa_{22}$ (SEQ ID NO : 4)

wherein the amino acids of the chain have the following meaning;
Xaa in position 1 is Arg, Lys, Asp or none
Xaa in position 2 is Trp, Gly, Lys or Arg,
Xaa in position 3 is Ile, Leu, Val or Met

Xaa in position 4 is Ile, Val or Leu
Xaa in position 5 Leu, Met, Val or Pro
Xaa in position 12 is Arg, Lys
Xaa in postion 13 is Met or Leu,
Xaa in position 15 is Ser, Cys or Gln,
Xaa in position 17 is Thr, Val, Ile, Ser or Ala,
Xaa in position 18 is Ser, Gly or Thr,
Xaa in position 21 is Asp, Glu, Cys or Gly,
Xaa in position 22 is Gly or none
wherein the sequence of SEQ ID NO : 4 comprises at least six consecutive amino acids and n = 0,1,2 or 3,

$Xaa_1\ Xaa_2 Xaa_3$ Pro Ile Pro $Xaa_7\ Xaa_8\ Xaa_9\ Xaa_{10} Xaa_{11}\ Xaa_{12}\ [Gly]_n\ Xaa_{13}\ Xaa_{14} Xaa_{15}$ $Xaa_{16}\ Xaa_{17}\ Xaa_{18}\ Xaa_{19}\ Xaa_{20}\ Xaa_{21}\ Xaa_{22}\ Xaa_{23}\ Xaa_{24}$ (SEQ ID NO : 9)

wherein Xaa in position 1 is Asn, Ser, Gly, His, Ala, Pro, Arg or none
Xaa in position 2 is Asn, Ala or Lys
Xaa in position 3 is Pro, Gln, Gly, Ile or Leu
Xaa in position 7 is Val or Ala
Xaa in position 8 is Gly or Lys
Xaa in position 9 is Glu, Asp, Lys, Phe or Thr
Xaa in position 10 is Ile, Met, Val or Leu
Xaa in position 11 is Tyr, Leu or none
Xaa in position 12 is Ser or none
Xaa in position 13 is Arg or none
Xaa in position 14 is Asp, Arg, Trp, Ala or none
Xaa in position 15 is Ile or none
Xaa in position 16 is Tyr or none
Xaa in position 17 is Lys or Arg
Xaa in position 18 is Arg, Lys or Asp
Xaa in position 19 is Trp or Gly
Xaa in position 20 is Ile, Met, Val, Gln or Ala
Xaa in position 21 is Ile, Val or Ala

Xaa in position 22 is Leu, Met or Val
Xaa in position 23 is Gly or Cys
Xaa in position 24 is Leu or none
wherein the sequence of SEQ ID NO : 9 consists of at least six consecutive amino acids and n = 1,2 or 3, and

Xaa_1 Xaa_2 Ile Ile Xaa_5 Xaa_6 Xaa_7 Xaa_8 Xaa_9 Leu Xaa_{11} $[Gly]_n$ $[Arg]_m$ Xaa_{12} Xaa_{13} Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17} Xaa_{18} Xaa_{19} Xaa_{20} Xaa_{21} Xaa_{22} Xaa_{23} Xaa_{24} Xaa_{25} (SEQ ID NO : 15)

wherein the Xaa in position 1 is Pro, Lys, Arg or none
Xaa in position 2 is Glu, Arg, Phe or Lys
Xaa in position 5 is Pro or Thr
Xaa in position 6 is Met, Thr or Nleu
Xaa in position 7 is Phe or Leu
Xaa in position 8 is Ser, Thr, Ala or Met
Xaa in position 9 is Ala, Glu or Leu
Xaa in position 11 is Ser or none
Xaa in position 12 is Ala, Arg or none
Xaa in position 13 is Ile, Leu or none
Xaa in position 14 is Ser, Ala, Leu or none
Xaa in position 15 is Tyr, Glu or Asp
Xaa in position 16 is Gly or Asp
Xaa in position 17 is Ala or Leu
Xaa in position 18 is Thr, Ile, Val, Leu or Asn,
Xaa in position 19 is Pro, Thr or Ser
Xaa in position 20 is Tyr, Phe, Nleu, His or Gln
Xaa in position 21 is Asp, Asn, Leu or Ala
Xaa in position 22 is Leu, Ile, Val or Asn
Xaa in position 23 is Asn, Tyr, Cys or Gly
Xaa in position 24 is Thr, Met, Ile, Ala, Val or none
Xaa in postion 25 is Gly or none
wherein the sequence of SEQ ID NO : 15 consists of at least six consecutive amino acids, n = 1, 2 or 3 and m= 0, 1, 2 or 3 independent of each other,

the terminal ends of the sequences may be free carboxyl- or amino groups, amides, acyls, acetyls or salts thereof,
two or more of the Cys residues may form part of an intrachain- or interchain disulphide binding, a -S-$(CH_2)_p$-S- or a - $(CH_2)_p$-bridge wherein p = 1-8 optionally intervened by one or more heteroatoms such as O, N and S and/or the said peptide sequences are immobilized to a solid support.

2. Peptide according to claim 1, characterized in that
the amino acid sequence of SEQ ID NO : 1 is selected from the groups of SEQ ID NO : 2 and SEQ ID NO : 3.

3. Peptide according to claim 1, characterized in that
the amino acid sequence of SEQ ID NO : 4 is selected from the groups of SEQ ID NO : 5, SEQ ID NO : 6, SEQ ID NO : 7 and SEQ ID NO : 8.

4. Peptide according to claim 1, characterized in that
the amino acid sequence of SEQ ID NO : 9 is selected from the groups of SEQ ID NO : 10 SEQ ID NO : 11, SEQ ID NO : 12, SEQ ID NO : 13 and SEQ ID NO : 14.

5. Peptide according to claim 1, characterized in that
the amino acid sequence of SEQ ID NO : 15 is selected from the groups of SEQ ID NO : 16, SEQ ID NO : 17, SEQ ID NO : 18, SEQ ID NO : 19 and SEQ ID NO : 20.

6. Antigen, characterized in that it comprises at least one peptide according to claim 1.

7. Antigen according to claim 6, characterized in that it comprises at least one peptide selected from each of the groups SEQ ID NO : 1, SEQ ID NO : 4, SEQ ID NO : 9 and SEQ ID NO : 15.

8. Vaccine composition, characterized in that it comprises an antigen according to claim 6 with a pharmaceutically acceptable diluent and optionally an adjuvant, carrier and/or vehicle and optionally additional immunostimulatory compound(s).

9. Vaccine composition according to claim 8, characterized in that it comprises at least four peptides selected from each of the groups of SEQ ID NO : 1, SEQ ID NO : 4, SEQ ID NO : 9 and SEQ ID NO : 15.

10. Vaccine composition according to claim 9, characterized in that it comprises the peptides of the SEQ ID NO : 3, SEQ ID NO : 6, SEQ ID NO : 11 and SEQ ID NO : 18.

11. Vaccine composition according to the claims 8-10 characterized in that the peptides are dissolved in a saline water solution and the optional immunostimulatory compound is a granulocyte macrophage growth factor.

12. Vaccine composition according to the claims 8-11 characterized in that the composition comprises an adjuvant selected from the group Monophosphoryl Lipid A (MPL®), Freund`s complete or incomplete adjuvant or aluminum hydroxyd.

13. A method of detecting antibodies, induced by a HIV or HIV-specific peptides or proteins, in a sample of body fluid characterized in that subjecting the said sample to an immunoassay, wherein the antigen(s) is/are selected from the peptides of the claims 1, 2, 3 , 4 and 5.

14. An immunoassay kit for the detection of antibodies, induced by a HIV or HIV-specific peptides or proteins, in a sample of body fluid, characterized in that the diagnostic antigen is a peptide of any one of the previous claims 1 to 5.

15. Antibody, characterized in that it is capable of selectively reacting with the antigen of the claims 6 and 7.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT (for all countries except US):
(A) NAME: Bionor A/S
(B) STREET: Strømdalsjordet 4, P.O.Box 1868 Gulset
(C) CITY: Skien

(E) COUNTRY: Norway
(F) POSTAL CODE (ZIP): N-3705
(G) TELEPHONE: +47 35 50 57 50
(H) TELEFAX: + 47 35 50 57 01

(i) INVENTOR AND APPLICANT (for US only) :
(A) NAME : Birger Sørensen
(B) STREET : Meierlia 3
(C) CITY : 3727 Skien
(D) COUNTRY : Norway

(ii) TITLE OF INVENTION: HIV Peptides, antigens, vaccine compositions, immunoassay and a method of detecting antibodies induced by HIV.

(iii) NUMBER OF SEQUENCES: 25

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: Windows 95
(D) SOFTWARE: Word 7.0

(v) CURRENT APPLICATION DATA:
Priority from NO 1999 1078 filed 4 March 2000
APPLICATION NUMBER:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(v) FRAGMENT TYPE : internal

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= " Xaa in position 1 is Lys or Arg

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= " Xaa in position 2 is Ala, Gly, Ser or Arg

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= " Xaa in position 3 is Leu or Met

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= " Xaa in position 4 is Gly or Arg

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= " Xaa in position 5 is Pro, Thr, Val, Ser, Gln or Ala
ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= " Xaa in position 6 is Gly, Ala, Lys, Arg, Gln or Glu
ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= " Xaa in position 8 is Thr or Ser

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= " Xaa in position 9 is Leu or Ile

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 14
(D) OTHER INFORMATION: /note= " Xaa in position 14 is Thr,Ser or Val

ix) FEATURE:
(A) NAME/KEY: Modified-site

(B) LOCATION: 15
(D) OTHER INFORMATION: /note= " Xaa in position 15 is Ala or Ser

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 16
(D) OTHER INFORMATION: /note= " Xaa in position 16 is Cys or Ser, optionally Cys forms part of a disulphide -bond

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 17
(D) OTHER INFORMATION: /note= " Xaa in position 17 is Gln or Leu.

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 18
(D) OTHER INFORMATION: /note= " Xaa in position 18 is Gly, Glu or Arg

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 20
(D) OTHER INFORMATION: /note= " Xaa in position 20 is Gly or Arg

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Xaa_1 Xaa_2 Xaa_3 Xaa_4 Xaa_5 Xaa_6 Ala Xaa_8 Xaa_9 Gln Thr Pro Trp Xaa_{14} Xaa_{15} Xaa_{16} Xaa_{17}
1 5 10 15

Xaa_{18} Val Xaa_{20}
20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 16

(D) OTHER INFORMATION: /note= " Optionally Cys in position 16 forms part of a disulphide bond

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Lys Ala Leu Gly Pro Gly Ala Thr Leu Gln Thr Pro Trp Thr Ala Cys Gln Gly Val Gly
1 5 10 15 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Ala Leu Gly Pro Ala Ala Thr Leu Gln Thr Pro Trp Thr Ala Ser Leu Gly Val Gly
1 5 10 15 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23-24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= " Xaa in position 1 is Arg, Lys, Asp or none

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2

(D) OTHER INFORMATION: /note= " Xaa in position 2 is Trp, Gly, Lys or Arg

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= " Xaa in position 3 is Ile, Leu, Val or Met

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= " Xaa in position is Ile , Val or Leu

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= " Xaa in position 5 is Leu, Met, Val or Pro

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(D) OTHER INFORMATION: /note= " Xaa in position 12 is Arg or Lys

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 13
(D) OTHER INFORMATION: /note= " Xaa in position 13 is Met or Leu

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(D) OTHER INFORMATION: /note= " Xaa in position 15 is Ser, Cys or Gln, optionally Cys forms part of a disulphide-bond

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 17
(D) OTHER INFORMATION: /note= " Xaa in position 17 is Thr, Val, Ile, Ser or Ala

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 18
(D) OTHER INFORMATION: /note= " Xaa in position 18 is Ser, Gly or Thr

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 21
(D) OTHER INFORMATION: /note= " Xaa in position 21 is Asp, Glu, Cys or Gly, optionally Cys forms part of a disulphide-bond

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 22
(D) OTHER INFORMATION: /note= " Xaa in position 22 is Gly or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11..12
(D) OTHER INFORMATION: /note= " optionally inserted Gly-bridge of 0,1,2 or 3 residues

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

$Xaa_1\ Xaa_2\ Xaa_3\ Xaa_4\ Xaa_5$ Gly Leu Asn Pro Leu Val $[Gly]_n\ Xaa_{12}\ Xaa_{13}$ Tyr Xaa_{15} Pro
1 5 10 15

$Xaa_{17}\ Xaa_{18}$ Ile Leu $Xaa_{21}\ Xaa_{22}$
20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 23
(D) OTHER INFORMATION: /note= " Cys in position 23 may forms part of a disulphide bridge

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Trp Ile Ile Pro Gly Leu Asn Pro Leu Val Gly Gly Gly Lys Leu Tyr Ser Pro Thr Ser Ile Leu
1 5 10 15 20

Cys Gly

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Trp Leu Leu Leu Gly Leu Asn Pro Leu Val Gly Gly Gly Arg Leu Tyr Ser Pro Thr Ser
1 5 10 15 20

Ile Leu Gly

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Ile Leu Leu Gly Leu Asn Pro Leu Val Gly Gly Gly Arg Leu Tyr Ser Pro Thr Ser Ile
1 5 10 15 20

Leu Gly

(2) INFORMATION FOR SEQ ID NO: 8

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

Arg Leu Leu Leu Gly Leu Asn Pro Leu Val Gly Gly Gly Arg Leu Tyr Ser Pro Thr Thr Ile
1 5 10 15 20
Leu Gly

(2) INFORMATION FOR SEQ ID NO: 9

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22-26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= " Xaa in position 1 is Asn, Ser, Gly His, Ala, Pro, Arg or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= " Xaa in position 2 is Asn , Ala or Lys

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= " Xaa in position 3 is Pro, Gln, Gly, Ile or Leu

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= " Xaa in position 7 is Val or Ala

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= " Xaa in position 8 is Gly, or Lys

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= " Xaa in position 9 is Glu, Asp, Lys, Phe or Thr

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 10
(D) OTHER INFORMATION: /note= " Xaa in position 10 is Ile, Met, Val or Leu

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11
(D) OTHER INFORMATION: /note= " Xaa in position 11 is Tyr, Leu or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(D) OTHER INFORMATION: /note= " Xaa in position 12 is Ser or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 13
(D) OTHER INFORMATION: /note= " Xaa in position 13 is Arg or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 14
(D) OTHER INFORMATION: /note= " Xaa in position 14 is Asp, Arg, Trp, Ala or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(D) OTHER INFORMATION: /note= " Xaa in position 15 is Ile or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 16
(D) OTHER INFORMATION: /note= " Xaa in position 16 is Tyr or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 17
(D) OTHER INFORMATION: /note= " Xaa in position 17 is Lys or Arg.

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 18
(D) OTHER INFORMATION: /note= " Xaa in position 18 is Arg, Lys or Asp

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19

(D) OTHER INFORMATION: /note= " Xaa in position 19 is Trp or Gly

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 20
(D) OTHER INFORMATION: /note= " Xaa in position 20 is Ile, Met, Val, Gln or Ala

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 21
(D) OTHER INFORMATION: /note= " Xaa in position 21 is Ile, Val or Ala

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 22
(D) OTHER INFORMATION: /note= " Xaa in position 22 is Leu, Met or Val

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 23
(D) OTHER INFORMATION: /note= " Xaa in position 23 is Gly or Cys

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 24
(D) OTHER INFORMATION: /note= " Xaa in position 24 is Leu or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12..13
(D) OTHER INFORMATION: /note= " optionally inserted Gly-bridge of 1,2 or 3 residues

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

$Xaa_1\ Xaa_2\ Xaa_3$ Pro Ile Pro $Xaa_7\ Xaa_8\ Xaa_9\ Xaa_{10}\ Xaa_{11}\ Xaa_{12}\ [Gly]_n\ Xaa_{13}\ Xaa_{14}\ Xaa_{15}$

1 5 10 15

$Xaa_{16}\ Xaa_{17}\ Xaa_{18}\ Xaa_{19}\ Xaa_{20}\ Xaa_{21}\ Xaa_{22}\ Xaa_{23}\ Xaa_{24}$

20

(2) INFORMATION FOR SEQ ID NO: 10

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 24
(D) OTHER INFORMATION: /note= " Cys in position 24 may forms part of a disulphide-bond

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10

Arg Asn Ile Pro Ile Pro Val Gly Asp Ile Tyr Gly Gly Gly Asp Ile Tyr Lys Arg Tyr Gln Ala
1 5 10 15 20
Leu Cys Leu

(2) INFORMATION FOR SEQ ID NO: 11

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11

Arg Ala Ile Pro Ile Pro Ala Gly Thr Leu Leu Ser Gly Gly Gly Arg Ala Ile Tyr Lys Arg Trp
1 5 10 15 20

Ala Ile Leu Gly
25

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

Ala Leu Pro Ile Pro Ala Gly Phe Ile Tyr Gly Gly Gly Arg Ile Tyr Lys Arg Trp Gln Ala Leu
1 5 10 15 20
Gly

2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

Lys Ile Pro Ile Pro Val Gly Phe Ile Gly Gly Gly Trp Ile Tyr Lys Arg Trp Ala Ile Leu Gly
1 5 10 15 20

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

Lys Ile Pro Ile Pro Val Gly Thr Leu Leu Ser Gly Gly Gly Arg Ile Tyr Lys Arg Trp Ala Ile
1 5 10 15 20
Leu Gly

(2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24-28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= " Xaa in position 1 is Pro, Lys, Arg or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= " Xaa in position 2 is Glu, Arg, Phe or Lys

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /note= " Xaa in position 5 is Pro or Thr

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(D) OTHER INFORMATION: /note= " Xaa in position 6 Met, Thr or Nle

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 7
(D) OTHER INFORMATION: /note= " Xaa in position 7 is Phe or Leu

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= " Xaa in position 8 is Ser, Thr, Ala or Met

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(D) OTHER INFORMATION: /note= " Xaa in position 9 is Ala, Glu or Leu

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11

(D) OTHER INFORMATION: /note= " Xaa in position 11 is Ser or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(D) OTHER INFORMATION: /note= " Xaa in position 12 is Ala, Arg or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 13
(D) OTHER INFORMATION: /note= " Xaa in position 13 is Ile, Leu or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 14
(D) OTHER INFORMATION: /note= " Xaa in position 14 is Ser, Ala, Leu or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(D) OTHER INFORMATION: /note= " Xaa in position 15 is Tyr, Glu or Asp

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 16
(D) OTHER INFORMATION: /note= " Xaa in position 16 is Gly or Asp

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 17
(D) OTHER INFORMATION: /note= " Xaa in position 17 is Ala or Leu

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 18
(D) OTHER INFORMATION: /note= " Xaa in position 18 is Thr, Ile, Val, Leu or Asn

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 19
(D) OTHER INFORMATION: /note= " Xaa in position 19 is Pro, Thr or Ser

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 20
(D) OTHER INFORMATION: /note= " Xaa in position 20 is Tyr, Phe, Nle, His or Gln

ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 21
(D) OTHER INFORMATION: /note= " Xaa in position 21 is Asp, Asn, Leu or Ala

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 22
(D) OTHER INFORMATION: /note= " Xaa in position 22 is Leu, Ile, Val or Asn

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 23
(D) OTHER INFORMATION: /note= " Xaa in position 23 is Asn, Tyr, Cys or Gly

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 24
(D) OTHER INFORMATION: /note= " Xaa in position 24 is Thr, Met, Ile, Ala, Val or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 25
(D) OTHER INFORMATION: /note= " Xaa in position 25 is Gly or none

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 23
(D) OTHER INFORMATION: /note= " optionally Cys in position 23 forms part of a disulphide-bond

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11...12
(D) OTHER INFORMATION: /note= " optionally a Gly-Arg bridge is inserted between Xaa 11 and 12, where n = 1, 2 and 3, and m independently of n is 0,1, 2 or 3.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

$Xaa_1\ Xaa_2$ Ile Ile $Xaa_5\ Xaa_6\ Xaa_7\ Xaa_8\ Xaa_9$ Leu $Xaa_{11}\ [Gly]_n\ [Arg]_m\ Xaa_{12}\ Xaa_{13}\ Xaa_{14}$

1 5 10

$Xaa_{15}\ Xaa_{16}\ Xaa_{17}\ Xaa_{18}\ Xaa_{19}\ Xaa_{20}\ Xaa_{21}\ Xaa_{22}\ Xaa_{23}\ Xaa_{24}\ Xaa_{25}$

15 20 25

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 24
(D) OTHER INFORMATION: /note= " Cys in position 24 optionally forms part of a disulphide-bond

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

Lys Phe Ile Ile Pro Nle Phe Ser Ala Leu Gly Gly Ala Ile Ser Tyr Asp Leu Asn Thr Nle
1 5 10 15 20
Leu Asn Cys Ile

(2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 26
(D) OTHER INFORMATION: /note= " Cys in position 26 optionally forms part of a disulphide-bond

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17

Lys Phe Ile Ile Pro Nle Phe Ser Ala Leu Ser Gly Gly Gly Ala Ile Ser Tyr Asp Leu Asn
1 5 10 15 20
Thr Phe Leu Asn Cys Ile Gly
25

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

Arg Phe Ile Ile Pro Nle Phe Thr Ala Leu Ser Gly Gly Arg Arg Ala Leu Leu Tyr Gly Ala
1 5 10 15 20
Thr Pro Tyr Ala Ile Gly
25

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19

Lys Ile Ile Pro Nle Phe Ser Ala Leu Gly Gly Gly Arg Leu Leu Tyr Gly Ala Thr Pro Tyr Ala
1 5 10 15 20
Ile Gly

(2) INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20

Arg Ile Ile Pro Nle Phe Thr Ala Leu Ser Gly Gly Gly Arg Leu Leu Tyr Gly Ala Thr Pro Tyr
1 5 10 15 20
Ala Ile Gly
25

(2) INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: dimeric peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: disulphide-bond between position 16 in SEQ ID NO : 2 and position 23 in SEQ ID NO : 5

(2) INFORMATION FOR SEQ ID NO:22

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: dimeric peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: disulphide-bond between position 16 in SEQ ID NO : 2 and position 16 in SEQ ID NO : 2
(D) OTHER INFORMATION: /note= "

(2) INFORMATION FOR SEQ ID NO:23

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: dimeric peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: disulphide-bond between position 23 in SEQ ID NO : 5 and position 23 in SEQ ID NO : 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 23
(D) OTHER INFORMATION: /note= " Cys in position 23 may forms part of a disulphide bridge

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Asn Ile Pro Ile Pro Val Gly Asp Ile Tyr Gly Gly Gly Asp Ile Tyr Lys Arg Tyr Gln Ala
1 5 10 15 20

Leu Cys Leu

(2) INFORMATION FOR SEQ ID NO:25

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: No

ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 23
(D) OTHER INFORMATION: /note= " Cys in position 23 optionally forms part of a disulphide-bond

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25

Trp Ile Ile Pro Nle Phe Ser Ala Leu Gly Gly Ala Ile Ser Tyr Asp Leu Asn Thr Nle
1 5 10 15 20
Leu Asn Cys Ile

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00075

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 14/16, C07K 16/10, A61K 39/21, A61K 39/295, G01N 33/569
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. Virol, Volume 73, No 1, January 1999, Lole KS et al, "Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination", page 152 - page 160, see locus AAD12087 a.a. 164-182	1,6-8,11-15
A	--	5
X	Nature, Volume 354, December 1991, Rodney E. Philips et al, "Human immunodeficiency virus genetic variation that can escape cytoxic T cell recognition", page 453 - page 459, table 1, page 24 res 255-271	1,6-8,11-15
A	--	4

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 July 2000	06 -07- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Hampus Rystedt/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00075

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9840744 A1 (BOEHRINGER MANNHEIM GMBH), 17 Sept 1998 (17.09.98), SEQ ID NO:14	1,6-8,11-15
A		4
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X	WO 9627013 A1 (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE-INSERM), 6 Sept 1996 (06.09.96), seqs 43-49, 71-73	1,6-8,11-15
A		5
	--	
X	WO 9428871 A1 (ENDOCON, INC.), 22 December 1994 (22.12.94), see page 12, lines 4, 5	1,6-8,11-15
A		4
	--	
A	Human Retroviruses and Aids, Bette Korber: "A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences", 1997, see page II-A-1-15	1-5
	--	
A	WO 9511255 A1 (AJINOMOTO CO., INC.), 27 April 1995 (27.04.95), page 10, line 7	1,4
	--	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00075

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1,6-8,11-15
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00075

A very large number of documents were found during the search for sequences belonging to the group defined by SEQ ID N:O 9. As the evaluation of all these documents would take an unreasonable amount of time, only a subset of the documents were evaluated and included in the search report. The search was then restricted to the fully specified sequences SEQ ID N:O 10-14. Consequently, all claims relating to SEQ ID N:O 9 are only partially searched.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NO 00/00075

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9840744 A1	17/09/98	AU 6727798 A	29/09/98
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